ABSTRACT

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Title of diploma thesis: Development of an analytical method for evaluation of pro-drug – boronyl salicylaldehyde isonicotinoyl hydrazone in biological material

High performance liquid chromatography is nowadays one of the most frequently used and progressive analytical method in pharmaceutical analysis. It can be used for both qualitative and quantitative analysis of complicated samples also in a biological matrix.

Iron is a vital element for all organisms. It's essential for a proper function of variety of crucial enzymes. On the other hand it's excess in the body is harmful and it is connected with progress of various pathological conditions. Hence iron chelation therapy is an effective tool to treat and protect living cells against injurious effect of iron.

Iron chelators were originally used to treat iron overload disease. However, thanks to their interesting antioxidative and antiproliferative properties, their therapeutic potential has been significantly widened. These compounds are currently investigated as promising drugs for the treatment of Parkinson's and Alzheimer's diseases, cancer, malaria, tuberculosis, etc.

Aroylhydrazone iron chelator salicylaldehyde isonicotinoyl hydrazone (SIH) is a small, lipophilic compound with low toxicity and significant antioxidative and cardioprotective effects both *in vitro* and *in vivo*. Unfortunately, this compound suffers from a very short biological half-life, which is a certain limitation for it's further development. The latest SIH analogue is it's pro-drug boronyl salicylaldehyde isonicotinoyl hydrazone (BSIH). BSIH is bioactivated inside the cells only in presence of oxidative stress. This feature provides a possibility to better focus a pharmacological effect of this chelator and avoid adverse effects of former compounds associated with an iron deprivation.

This study is focused on development of HPLC method for simultaneous analysis of SIH and it's pro-drug BSIH in biological materials (plasma, cell medium). The best

separation was acquired on a HPLC column Zorbax Bonus-RP (150x3 mm, particle 3.5µm) protected by guard column. Mobile phase was composed of 0.02 M phosphate buffer (pH 6.0) and a mixture of methanol and acetonitrile (40:60, v/v), in a ratio of 60:40 (v/v). A flow rate was set to 0.3 ml/min and detection wavelength was 297 nm. Retention times of 4.5 min for BSIH, 14.2 min for SIH and 12.6 min for internal standard o-108 were obtained.

Plasma samples were treated by precipitation with methanol, while the cell medium was simply diluted with the same solvent. Linearity of the method for determination of SIH and BSIH was proven within the range from 5 to 40 µg/ml and 10 to 50 µg/ml for cell medium and plasma, respectively. Precision and accuracy of the method were assessed only for plasma and reached acceptable values.